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# Effects of ocean acidification on algae growth and feeding rates of juvenile sea urchins

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ABSTRACT

The recent decrease in seawater pH has stimulated a great deal of research on the effects of ocean acidification on various organisms. Most of these studies have mainly focused on the direct effects of acidification on organisms. However, the effects on ecological interactions have been poorly studied. In this paper we have focused on determining the effects of acidification on feeding rates of two species of sea urchins, *Paracentrotus lividus* and *Diadema africanum* through laboratory experiments. Nine algae species were reared under two pH treatmens (ph = 8.1 vs. pH = 7.6) for 10 days. We evaluated possible changes in calcification rates, growth and internal structure. Then these algae were offered to juvenile sea urchins for 7 days, evaluating the consumption rates of juvenile sea urchins under these different pH conditions. The algae reared in the control treatment showed higher growth rates and concentration of calcium carbonate, however no internal structural changes were observed in any algae. Juvenile *Paracentrotus lividus* showed higher consumption rates on algae previously subjected to pH 7.6 than on algae reared under control conditions and between algae species in low pH.The algae most consumed were *C. liebetruthii, C. abies-marina* and *C. elongata* by *P. lividus* juveniles from low pH treatment. However in *D. africanum* the feeding rates were similar between treatments. This study demonstrated the negative effects of low pH on various species of algae in growth, and indirectly the increase in herbivory rates of juvenile sea urchins on algae reared under low pH.

## 1. Introduction

In recent decades, the progressive increase of $CO_2$ in the atmosphere has led to increased $pCO_2$ in the oceans, triggering a series of changes in the chemistry of seawater. Excess $CO_2$ on contact with sea water dissociates into carbonic acid ($H_2CO_3$) and bicarbonate (HCO3). Bicarbonate dissociates into $CO_3^{-2}$ and $H^+$, which leads to a decrease in pH. Predictions suggest that by the year 2100 oceans will reach an average of 7.7 units (IPCC, 2013). However, by the end of the 23rd century, there may have been a decrease of 0.7 units (Caldeira and Wicket, 2003; Orr et al., 2005). These changes in pH chemistry will have the greatest impacts on those organisms that use forms of dissolved carbon (DIC) to photosynthethise or to form their calcareous structures, such as algae, corals, gastropods and sea urchins (Gattuso et al., 1999; Kleypas and Langdon, 2006; Anthony et al., 2008; Andersson et al., 2011; Koch et al., 2013).

It is especially important to understand how habitat-forming species are being affected by ocean acidification. Algae are ecosystem engineers of benthic systems but their biomass largely depends on herbivore pressure. The hervibore impact on marine benthic primary producers is intense, reducing producer abundance by 68% on average compared with 49–59% estimated from previous analysis of aquatic systems (Gruner et al., 2008), and consistently higher than estimates form terrestrial vegetation (Cebrian, 1999). For instance, ocean acidification can change the biochemical composition and nutritional quality of primary producers (Rossoll et al., 2012). These changes can affect the resources utilized in herbivores growth, altering species composition and dominance of communities in future climate change scenarios (Falkenberg et al., 2013; Poore et al., 2013). In addition to direct effects on herbivore abundances or nutritional quality of primary producers, climatic stressors will affect the strength of algae-herbivore interaction by changes in the susceptibility of algae tissues to herbivore.

Most research into ocean acidification has focused on the 'fitness' and physiological tipping points of certain species through laboratory conditions, but only a few studies have recently begun to evaluate interactions among organisms, either in their natural environments (Johnson et al., 2012; Porzio et al., 2011; Uthicke et al., 2016), using mesocosm experiments (Alstenberg et al., 2013; Tomas et al., 2015) or

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in laboratory conditions (Diaz-Pulido et al., 2011; Doropoulos et al., 2012; Asnaghi et al., 2013; Poore et al., 2013, 2016; Hernán et al., 2016; Rodríguez et al., 2017a; Jiménez- Ramos et al., 2017). Due to the complex interactions that occur in the natural environment, a greater effort is needed to study interactions between multiple species, including relationships involving competition, predation and/or herbivory (Wernberg et al., 2012; Connell et al., 2013).

Macroalgae show different responses to ocean acidification (Hall-Spencer et al., 2008; Nelson, 2009; Martin and Gattuso, 2009; Connell and Russell, 2010; Cornwall et al. 2011, 2017; Poore et al., 2013). Some effect has been a shift in the distribution of species (Harley et al., 2012; Bijma et al., 2013) following physiological tolerance limits that enable species survival at certain environmental conditions, but also negative physiological effects on the growth of calcifying algae are often reported (Diaz-Pulido et al., 2011; Kroeker et al., 2013; Hofmann and Bischof, 2014). The effects on non-calcifying algae are not well understood (Connell and Russell, 2010; Harley et al., 2012; Wernberg et al., 2012), however, a recent study by Cornwall et al. (2017) found varying responses of algae depending on their specific DIC acquisition mechanisms. Other studies highlight the changes in phenols content or in structural content (C:N) in seagrasses or algae species under ocean acidification conditions (Rossoll et al., 2012; Tomas et al., 2015; Jiménez-Ramos et al., 2017).

Sea urchins are key herbivores in coastal areas (Ruitton et al., 2000; Hernández et al., 2008; Sangil et al., 2014). Urchin population outbreaks often occur due the overfishing of their specific predators (Sala et al., 1998; Clemente et al., 2010). These increases in sea urchin densities can cause dramatic changes in the structure and functioning of ecosystems, limiting algal growth by intense grazing, promoting a shift in communities from predominantly erect macroalgae to crustose algae and urchin barrens (Hernández et al., 2008; Ling et al., 2015). Effects of ocean acidification on sea urchins has been broad studied, focused in early stages principally, however few studies have evaluated the effects of ocean acidification on algae-sea urchins interactions (Johnson and Carpenter, 2012; Asnaghi et al., 2013) or seagrass-sea urchins interactions (Burnell et al., 2013; Tomas et al., 2015; Johnson et al., 2012).

In this study, we have focused on evaluating the effects of ocean acidification on the feeding rates upon several algae species of two sea urchin species, *Paracentrotus lividus* and *Diadema africanum*, key herbivores in rocky bottom ecosystems of the Canary Islands (Hernández et al., 2013). *Paracentrotus lividus* is common in shallow subtidal communities, with densities between 1 and 2 individuals/$m^2$ (Girard, unpublished data). However, *D. africanum* is the most abundant herbivore in rocky reef communities, with desities of 10 individuals/$m^2$ (Hernández et al., 2013). Both sea urchins species can coexist in the same habitat, juvenile stages predominantly. The main objective was to detect any alteration in the feeding rates of the chosen echinoids under low pH and control pH conditions as consequence of a change in algae palatability. Future ocean acidification conditions could change the algae tissues, altering their palatability, and therefore we have also studied the calcification and internal structure of nine species of algae consumed in other experiments by sea urchin species chosen (Rodríguez et al., 2017a). We hypothesized: (1) that algae species more vulnerable to acidification will be more susceptible to herbivory, and (2) there will be significant changes in consumer preferences and/or feeding rates.

## 2. Materials and methods

### 2.1. Study organisms and sites

Two species of echinoids (*Paracentrotus lividus* and *Diadema africanum*) and nine species of algae (*Cladophora liebetruthii*, *Colpomenia sinuosa*, *Cystoseira abies-marina*, *Dictyota dichotoma*, *Lobophora variegata*, *Padina pavonica*, *Corallina elongata*, *Halopteris socoparia* and *Stypopodium zonale*) were chosen for experimental procedures. The selected algae were those that showed higher and lower feeding rates during a previous study with juveniles of *P. lividus* and *D. africanum* (Rodríguez et al., 2017b). For *D. africanum*, the algae chosen were: *Cladophora liebetruthii*, *Colpomenia sinuosa*, *Coralina elongata*, *Cystoseira abies-marina*, *Dictyota dichotoma*, *Lobophora variegata* and *Padina pavonica*; and for *P. lividus*: *C. liebertruthii*, *C. elongata*, *C. abies-marina*, *Halopteris socoparia*, *L. variegata*, *P. pavonica* and *Stypopodium zonale*. All specimens were colleted at Tenerife Islands (Canary Islands): algae were collected by snorkelling at Punta del Hidalgo (28° 34′ 23.4″ N, 16° 32′ 47.87″ W) between April and October 2013; *P. lividus* and *D. africanum* juveniles were collected at Abades (28′31.05″ 28° N, 16° 26′ 12.49″ W) and Punta Prieta (28° 16′ 20.33″ N, 16° 23′ 4.43″ W) in October 2013 and April 2013, respectively. Both localities show high recruitment rates of both species for these periods (Girard et al., 2008; Hernández et al., 2010).

### 2.2. Experiment design

Two consumption experiments at different pH treatments were performed: (1) with juvenile *P. lividus* in October 2013 and (2) with juvenile *D. africanum* in April 2013. In both cases, the organisms were subjected to two pH treatments: control pH (pH = 8.1), and low pH (pH = 7.6) simulating forecasts for the forthcoming centenary (IPCC, 2013). Previous to the experiments, juvenile sea urchins were acclimated for 72 h and fed *ad libitum* with *Ulva compressa*. The system consisted in two tanks of 300 L interconnected in each treatment; one tank was header tank where pH was adjusted although a $CO_2$ injection system (AQUAMEDIC) in low pH treatment and the temperature was constant at 20 ± 0.3 °C using coolers (ECHEIM AQUATIC, 50 W, with a precision ± 0.5 °C) The other tank was the support where the beakers were placed.

Five replicates of each algae species were used per pH treatment. Three additional replicates for each algae species were used as methodological controls without sea urchins. These were necessary to estimate changes of algal biomass in the absence of herbivory (Roa, 1992). Isolated experimental containers were immersed in a tapwater bath to maintain a constant temperature of 20 ± 0.3 °C. The seawater used in the beakers was filtered through active carbon filters, 200 μm polyamide mesh and sterilized using UV filters in header tank. Treatment of low pH was maintained using a computerized system (AquaMedic) that regulated pH by bubbling pure $CO_2$ directly into the water, to a resolution of ± 0.1 pH units. Temperature, pH and salinity were measured daily during the experiment. Temperature and pH were measured with a sensor (Metrohm mobile meter with a Primatrode NTC IP pH electrode and temperature sensor). The salinity was registered using a salinometer (COND 315i). The alkalinity was measured by titration once a week in each treatment. The other chemical parameters of seawater: $pCO_2$, and the saturations of calcite (Ωc) and aragonite (Ωa) were calculated from the total alkalinity (TA) and pH using the CO2SYS software (Lewis and Wallace, 1998). Calculations were based on the constants K1 and K2 from Mehrbach et al. (1973), modified by Dickson and Millero (1987).

A piece of each alga, which ranged between 0.084 g and 1.498 g, was placed in each experimental beaker. The algal piece was previously centrifuged with a hand centrifuge to remove excess water and weighed on a precision balance ( ± 0.001 g). After ten days of culture in pH conditions, algae from each replicate were weighed again and exposed to juvenile sea urchins. The horizontal test diameter of each echinoid was previously registered using a digital caliper ( ± 0.01 mm). For 7 days, juvenile sea urchins were left to feed on each of the species of

**Table 1**
Chemical seawater parameters during the feeding rate experiment for juvenile sea urchins A: *P. lividus* and B. *D. africanum*. The values showed are means ( ± SD) of total pH ($pH_T$), total alkalinity (TA) and temperature for 17 days. The rest of the values: the partial pressure $CO_2$ ($pCO_2$), calcite saturation (Ωc), aragonite saturation (Ωa) were calculated using CO2SYS.

| Sp | $pH_T$ | $pCO_2$(µatm) | $A_T$ (mmol $kg^{-1}$) | $CO_3^{-2}$ (mmol $Kg^{-1}$) | $HCO_3^-$(mmol $Kg^{-1}$) | $\Omega_c$ | $\Omega_a$ | T (ºC) |
|---|---|---|---|---|---|---|---|---|
| **A.** *P. lividus* | 7.60 ± 0.1 | 1349.0 ± 82 | 2.507 ± 0.012 | 0.086 ± 0.020 | 2.301 ± 0.023 | 1.99 ± 0.21 | 1.29 ± 0.12 | 19.4 ± 0.4 |
| | 8.10 ± 0.1 | 413.2 ± 42 | 2.854 ± 0.011 | 0.258 ± 0.089 | 2.237 ± 0.061 | 6.12 ± 0.54 | 3.97 ± 0.21 | 19.5 ± 0.3 |
| **B.** *D. africanum* | 7.60 ± 0.1 | 1349.0 ± 82 | 2.507 ± 0.012 | 0.086 ± 0.020 | 2.301 ± 0.023 | 1.99 ± 0.21 | 1.29 ± 0.12 | 19.4 ± 0.4 |
| | 8.10 ± 0.1 | 413.2 ± 42 | 2.854 ± 0.011 | 0.258 ± 0.089 | 2.237 ± 0.061 | 6.12 ± 0.54 | 3.97 ± 0.21 | 19.5 ± 0.3 |

algae selected, so that the complete experiment lasted 17 days. At the end, each experimental algae piece was weighed following the above mentioned methodology. To estimate the consumption rate of each of the algae species by sea urchins, we used the following equation:

$$Ctot = Tt*\left(\frac{Cf}{Ct}\right) - Tf$$

Where $C_{tot}$ is total consumption in grams (g), $T_i$ is the initial algae weight (g) and $T_f$ the final algae weight in the treatment with sea urchins, $C_i$ is the initial algae weight and $C_f$ the final algae weight in the control treatment (without urchins).

Consumption rates of each of the sea urchin species were analysed by two-way ANCOVAs performed by permutations (Anderson, 2004). In the models, 'treatment pH′ was treated as a fixed factor with 2 levels: low pH (7.6) and control pH (8.1); and 'algae' as a fixed factor with 7 levels. In the case of the sea urchin *P. lividus,* these levels were *C. liebetruthii*, *C. abies-marina*, *C. elongata*, *H. scoparia*, *L. variegata*, *P. pavonica* and *S. zonale*; in the case of *D. africanum* the levels were *C. liebetruthii*, *C. sinuosa*, *C. abies-marina*, *D. dichotoma*, *C. elongata*, *L. variegata* and *P. pavonica*. Although a very narrow range of urchin sizes was used for the experiments, the horizontal test diameter was treated as a covariate, in order to control any influence of urchin size on consumption rates.

The algae from the control treatment were used to estimate algal growth under two pH treatments. We had 3 replicates per algae species and pH treatment. Each specimen was weighed at the start of the experiment, at ten days and at the end of experiment (after 17 days). To remove excess surface water from the algae, they were centrifuged for 10 s using a hand centrifuge. The relative growth rate (RGR) of each species was estimated using the Gao et al. (1993) formula:

$$RGR = \frac{\left(100\ Ln\left(\frac{N_t}{N_0}\right)\right)}{t}$$

Where $\mathbf{N_t}$ is the final wet weight in a certain period (10 or 17 days) and $\mathbf{N_0}$ is initial wet weight. The variable **t** is the exposure time at the experimental conditions (10 or 17 days).

The relative growth rate of the algae was analysed through a 3-way ANOVA by permutations (Anderson, 2004), with 3 fixed factors: 'Treatment pH′, with 2 levels: Low pH (pH = 7.6) *vs.* Control pH (pH = 8.1); 'Algae', with 9 levels: *C. liebetruhii*, *C. sinuosa, C. abies-marina, C. elongata, D. dichotoma, H. scoparia, L. variegata, P. pavonica and S. zonale*; and 'exposure time', with 2 levels: 10 days and 17 days of experiment.

A variable amount of each of the experimental species of algae (1.178–6.607 g) was deposited in small rigid mesh bags (mesh opening: 2 mm) on each seawater table with the corresponding pH treatment. We used 3 replicates per algae species and pH treatment. These replicates were weighed at the beginning and end of the experimental period with a precision balance ( ± 0.001 g). Then, each replicate was dried in an oven at 60 °C for 48 h to obtain the dry weight. Algae were decalcified with hydrochloric acid (1 N) overnight. After decalcification, the algae were dried again in the oven for 36 h until their weight stabilized, following the methodology of Martone (2010) and Johnson et al. (2012). $CaCO_3$ content (%) was calculated for each species as the difference between un-decalcified and decalcified dry weights of algae (Martone, 2010).

To evaluate differences in calcification of algae, the percentage of $CaCO_3$ was analysed with a two-way ANOVA performed by permutations (Anderson, 2004) with 2 fixed factors: 'treatment pH′, each with two levels: Low pH (7.6) vs. Control pH (8.1); and 'algae', with 8 levels: *C. liebetruhii*, C. *elongata*, *C. abies-marina*, *D. dichotoma*, *H. scoparia*, *L. variegata*, *P. pavonica* and *S. zonale*. The alga *C. sinuosa* could not be included in the analysis because it is seasonal and could not be collected for the experiment.

Algae from each treatment were cut transversally under a binocular microscope with a scalpel before and after the experiment, to detect whether the internal structure of each of the species was modified according to pH treatment. We followed the methodology by Bradassy et al. (2013) to study the internal structure. Cuts were photographed using a camera-associated LEICA microscope. Through the images, we could observe any internal structure changes after the maintenance at the two pH treatments.

## 3. Results

### 3.1. 3.1Seawater chemistry

The seawater parameters measured during the experiment are shown in Table 1. The mean values ( ± SD) for the control treatment were 8.1 ± 0.1 for pH, and 413.2 ± 43 µatm for partial pressure ($pCO_2$). For the low pH treatment, they were 7.6 ± 0.1 and 1234.5 ± 83 µatm respectively. The temperature was kept constant at 20 °C and 19.5 °C in the control treatment and low pH treatment, respectively.

### 3.2. Effects of pH on algae palatability

For *Paracentrotus lividus*, algae palatability varied between pH treatments ($F = 17.47$; $p < 0.001$) and between algae species ($F = 2.40$; $p < 0.05$) (Table 2 and Fig. 1). Algae reared in low pH showed higher feeding rates by sea urchins than those reared in control pH. However, the feeding rates were different between algae species, regardless of the pH treatment considered. The algae *C. liebetruthii, C.*

**Table 2**
Results of two-way permutational ANCOVA comparing feeding rates by juvenile *Paracentrotus lividus* between two pH treatments (pH = 8.1 vs. pH = 7.6) and between 7 species of algae (*C. liebetruthii* vs. *C. sinuosa* vs. *C. elongata* vs. *C. abies-marina* vs. *D. dichotoma* vs. *L. variegata* vs. *P. pavonica*).

| Source of variation | df | SS | MS | F | p (perm) |
|---|---|---|---|---|---|
| *Size(S)* | 1 | 0.001 | 0.009 | 1.99 | 0.163 |
| *pH Treatment(Tr)* | 1 | 0.095 | 0.095 | 17.47 | **0.001** |
| *Algae (Al)* | 6 | 0.078 | 0.013 | 2.40 | **0.048** |
| *S* Tr* | 1 | 0.001 | 0.001 | 0.06 | 0.796 |
| *S*Al* | 6 | 0.047 | 0.079 | 1.45 | 0.230 |
| *Tr*Al* | 6 | 0.022 | 0.037 | 0.69 | 0.658 |
| *S*Tr*Al* | 6 | 0.012 | 0.002 | 0.38 | 0.860 |
| *Residual* | 42 | 0.228 | 0.005 | | |
| *Total* | 69 | 0.495 | | | |

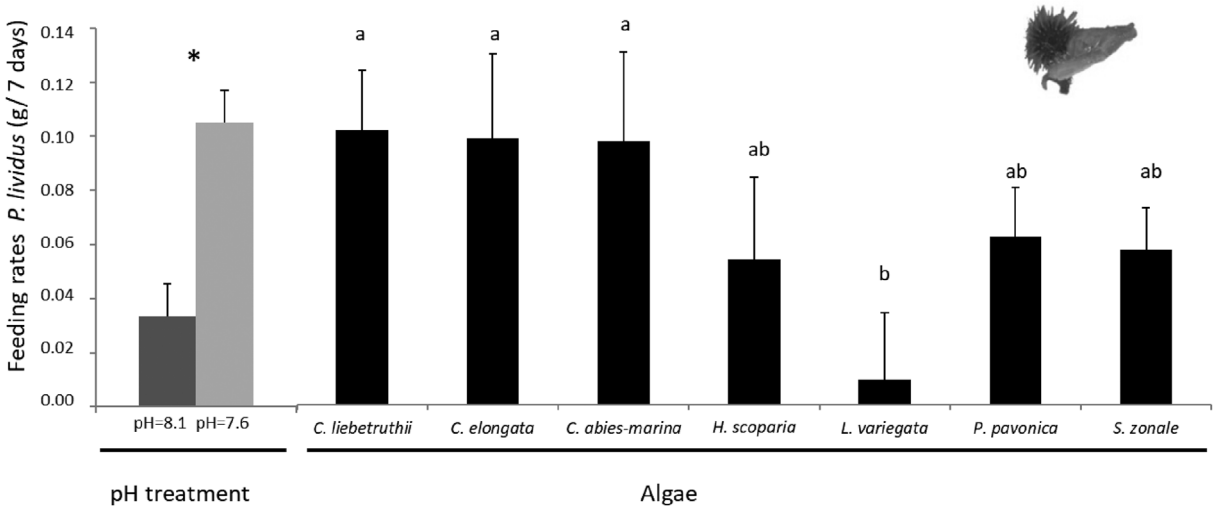


**Fig. 1.** Mean ( ± SD) feeding rates (g) of different algae species by juvenile sea urchins *Paracentrotus lividus* and for each pH treatment (Control pH = 8.1 and Low pH = 7.6) in the feeding experiments conducted in the laboratory. Differences significatives are showed with asterisk (*) between pH treatments and with different letters between algae species.

**Table 3**
**(A)** Results of the two-way permutational ANCOVA comparing feeding rates of *Diadema africanum* juveniles in two pH treatments (pH = 8.1 vs. pH = 7.6) and between algae (*C. liebetruthii* vs. *C. sinuosa* vs. *C. elongata* vs. *C. abies-marina* vs. *D. dichotoma* vs. *L. variegata* vs. *P. pavonica*). (B) *A posteriori* pairwise comparisons of the significant interaction of factors 'pH Treatment x Algae'. Significant results ($p < 0.05$) are shown in bold.

| A. Source of variation | df | SS | MS | Pseudo-F | p (perm) |
|---|---|---|---|---|---|
| *Size(S)* | 1 | 0.060 | 0.060 | 5.34 | **0.026** |
| *pH Treatment (Tr)* | 1 | 0.004 | 0.004 | 0.38 | 0.542 |
| *Algae (Al)* | 6 | 0.372 | 0.062 | 5.52 | **0.001** |
| *S* Tr* | 1 | 0.001 | 0.017 | 1.50 | 0.230 |
| *S* Al* | 6 | 0.211 | 0.023 | 2.07 | 0.082 |
| *Tr*Al* | 6 | 0.211 | 0.035 | 3.13 | **0.014** |
| *S* Tr*Al* | 6 | 0.048 | 0.008 | 0.71 | 0.633 |
| *Residual* | 42 | 0.472 | 0.011 | | |
| *Total* | 69 | 1.325 | | | |

| **B. Pairwaise** | | **Treatment** | **T** | **p (MC)** |
|---|---|---|---|---|
| *Algae* | *C. liebetruthii* | 7.6 vs. 8.1 | 1.240 | 0.257 |
| | *C. sinuosa* | 7.6 vs. 8.1 | 1.469 | 0.193 |
| | *C. elongata* | 7.6 vs. 8.1 | 1.389 | 0.209 |
| | *C. abies-marina* | 7.6 vs. 8.1 | 0.040 | 0.968 |
| | *D. dichotoma* | 7.6 vs. 8.1 | 3.343 | **0.015** |
| | *L. variegata* | 7.6 vs. 8.1 | 1.602 | 0.156 |
| | *P. pavonica* | 7.6 vs. 8.1 | 0.993 | 0.358 |

*abies-marina* and *C. elongata* showed higher consumption rates by *P. lividus* than *L. variegata,* which was the least consumed species (Supplementary material 1 and 2). Sea urchin size did not show a significant effect on the consumption rates of the algae species (Table 2).

For *Diadema africanum*, analysis showed a significant interaction between pH and algae species, which means that the effect of pH on feeding rates varied between algae species (Table 3A). Pairwise analysis only showed significant differences between pH levels for the alga *D. dichotoma*, where sea urchins had a higher consumption rate of algae from the control treatment than algae reared at pH 7.6 ($F = 3.343$; $p < 0.05$). For the other algae there were no significant differences in feeding rates according to pH. However, there was a trend towards higher consumption of algae in control treatment, except for *C. liebetruthii* and *C. sinuosa*. In both the latter, the trend was reversed, with higher consumption by *D. africanum* in the low pH treatment (Table 3B and Fig. 2). In addition, a significant effect of *D. africanum* sizes over consumption rates was detected (Table 3A).

### 3.3. Effects of pH on algae growth

The growth of algae varied between pH treatments ($F = 9.10$; $p < 0.01$), between algae species ($F = 23.24$, $P < 0.001$) and between exposure times ($F = 22.618$, $p < 0.001$) (Table 4). The algal growth rate was generally higher in the control than in the low pH treatment (Fig. 3A). Moreover, the algae species differ from each other in their growth rates, regardless of pH and exposure time. *Stypopodium zonale* showed faster growth rates than *C. liebetruthii* and *H. scoparia*, which showed slow growth, followed by *C. sinuosa*, *D. dichotoma* and *L. variegata*. The algae *C. elongata*, *C. abies-marina* and *P. pavonica* had intermediate growth rates (Fig. 3B and Supplementary material 3–4). The algae had higher growth rates during the first 10 days of experiment than in the last 7 days where a loss of mass was observed (Fig. 3B).

### 3.4. Effects of pH on algae calcification

The calcium carbonate content in dry weight varied significantly between pH treatments ($F = 24.07$, $p < 0.001$) and between algae species considered ($F = 15.98$; $p < 0.001$) (Table 5). Algae maintained in the control treatment had a higher percentage of calcium carbonate than those grown in low pH conditions (see Fig. 4). The algae that had a higher percentage of calcium carbonate in dry weight were *P. pavonica*, *D. dichotoma*, *C. liebetruthii*, followed by *C. abies-marina*, *L. variegata*, *H. scoparia* and *S. zonale* (See Supplementary Material 5). In calcareous algae (*C. elongata and P. pavonica*) the percentages of $CaCO_3$ in the low pH were 5.93 and 31.29%, respectively, and in the control treatment 22.07 and 53.52% (Supplementary Material 6).

### 3.5. Effects of pH on algae structural internal

No differences in the internal structure of algae were observed between pH treatments. Only a loss in $CaCO_3$ was observed on *P. pavonica* thallus surface cultured at the low pH treatment (see Supplementary material 7).

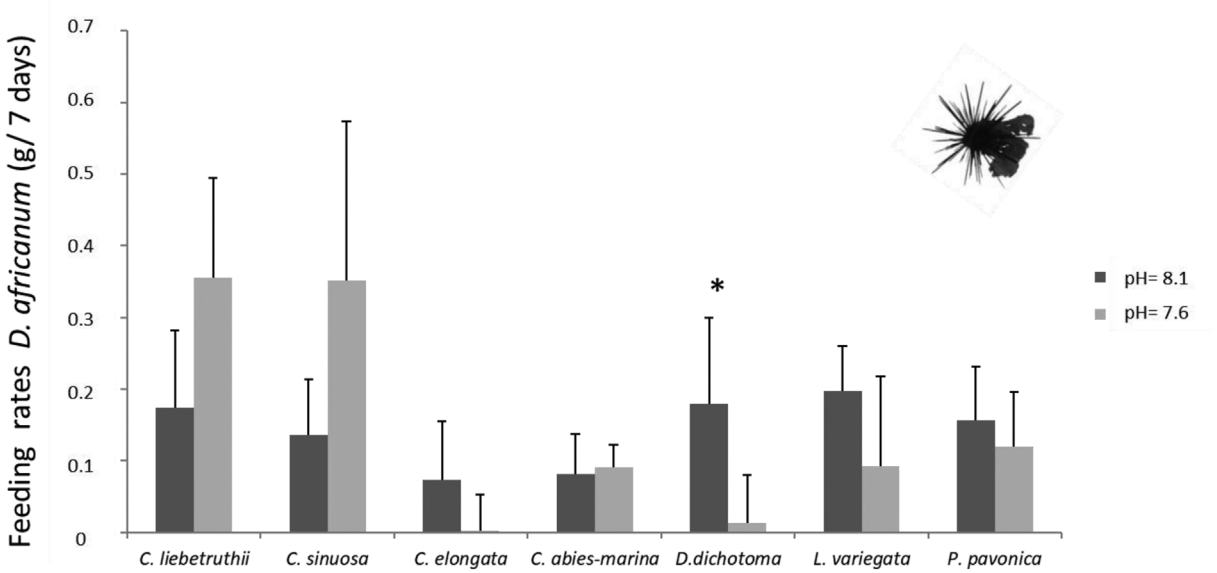


**Fig. 2.** Mean ( ± SD) feeding rates (g) of different algae species by juvenile *D. africanum* cultured at different pH treatments (Control pH = 8.1 and low pH = 7.6) in the feeding experiments conducted in the laboratory. (*) = $p < 0.05$.

**Table 4**
Results of three-way permutational ANOVA comparing algal growth between pH treatments (pH = 8.1 vs. pH = 7.6), algae species (*C. liebetruthii*, *C. sinuosa*, *C. elongata*, *C. abies-marina*, *D. dichotoma*, *H. scoparia*, *L. variegata*, *P. pavonica* and *S. zonale*) and exposure time (10 vs. 17 days).

| Source of variation | df | SS | MS | Pseudo-F | p (perm) |
|---|---|---|---|---|---|
| *pH Treatment(Tr)* | 1 | 14.503 | 14.503 | 9.10 | **0.004** |
| *Algae(Al)* | 8 | 296.38 | 37.047 | 23.26 | **0.001** |
| *Time (T)* | 1 | 36.056 | 36.056 | 22.62 | **0.045** |
| *Tr* Al* | 8 | 17.471 | 2.1838 | 1.37 | 0.218 |
| *Tr* T* | 1 | 0.1846 | 0.1846 | 0.12 | 0.741 |
| *Al*T* | 8 | 0.022 | 1.4115 | 0.88 | 0.531 |
| *Tr*Al*T* | 8 | 0.012 | 1.3591 | 0.85 | 0.566 |
| *Residual* | 102 | 162.6 | 1.5941 | | |
| *Total* | 137 | 552.08 | | | |

## 4. Discussion

Many studies have focused in the direct effects of ocean acidification on marine species, however some studies highlight the importance of indirects effects of ocean acidification on marine species (Pavia & Toth, 2000; Harley et al., 2012; Poore et al., 2013; Gutow et al., 2014; Jiménez Ramos et al., 2017). In this study we highlight the indirect effects of ocean acidification on feeding rates of two species of sea urchins. Juvenile *Paracentrotus lividus* showed an increasing general algal consumption at low pH. This response to ocean acidification has been previously reported for other species, such as juvenile *Acanthaster planci* (Kamya et al., 2016). However, the response of *Diadema africanum* was different; its feeding rate was similar in both pH treatments assesed, except on the alga *D. dichotoma,* which showed higher consumption rates by sea urchins under control pH conditions. These results show alterations in patterns of herbivory due to ocean acidification, as previously reported for some other species (Vergés et al., 2014; Tomas et al., 2015; Poore et al., 2016), and contrasting effects among sea urchin species.

*Paracentrotus lividus* is a robust species that inhabits subtidal and intertidal zones; in this sense it is possibly well adapted to strong pH fluctuations (García et al., 2018a). It has been suggested that this ability is an advantage to cope with future scenarios of ocean acidification (García et al., 2018a). However, *D. africanum* is exclusively in subtidal zone where the daily pH fluctuations are not pronounced. This low pH fluctuations could be limiting the capacity of *D. africanum* to adaptation to ocean acidification conditions.

The increase in consumption rates under acidification observed in *P. lividus* can be explained by (1) higher metabolic energy cost of living at low pHs (Calosi et al., 2013), (2) low nutritional quality of algae, as also found in seagrasses (see Cruz Rivera and Hay, 2000; Xu et al., 2010; Falkenberg et al., 2014), or (3) alterations in chemical and structural traits due to ocean acidification (Hemmi and Jormalainen, 2002), or a combination of both types of traits in the algae. Such alterations could modify the algae palatability through changes in tissues algae and then their feeding rates, as previously observed in terrestrial plants (Stiling and Cornelissen, 2007), kelp (Swanson and Fox, 2007) and seagrass (Tomas et al., 2015; Johnson et al., 2012). However, the similar feeding rates between pH treatments in juvenile *D. africanum* show different responses between sea urchin species. In juvenile stages, this species is attracted by chemical cues and by algal structure (see Rodríguez et al., 2017b). The low feeding rate observed in *Diadema* in low pH treatment suggests possible changes in tissue toughness or loss of chemical defences in algae.

In this study we have explored the third above mentioned hypothesis, studying the changes in growth and internal structure of algae under acidification, and how these changes could affect algae-sea urchin interaction by changes to the susceptibility of algae tissues to herbivory. However, our results did not show any relation with changes in internal structure of studied species, but the secondary metabolites were not studied in this experiment. Some studies show the importance of chemical metabolites as chemical defences under ocean acidification condition, increasing the susceptibility of alga to herbivory by changes in the production of the defensive secondary metabolites or concentration of defensive phenolics in seagrass or algae (Arnold et al., 2012; Swanson and Fox, 2007). Negative effects of ocean acidification on growth have been observed in other seaweed species as well. For example, growth of *Hypnea musciformis* (Israel and Hopi, 2002), *Lobophora papenfussii* (Diaz-Pulido et al., 2011), *Porphyra linearis* (Israel et al., 1999) or *Zinaria diesingiana* and *Halopeltus australis* (Poore et al.,

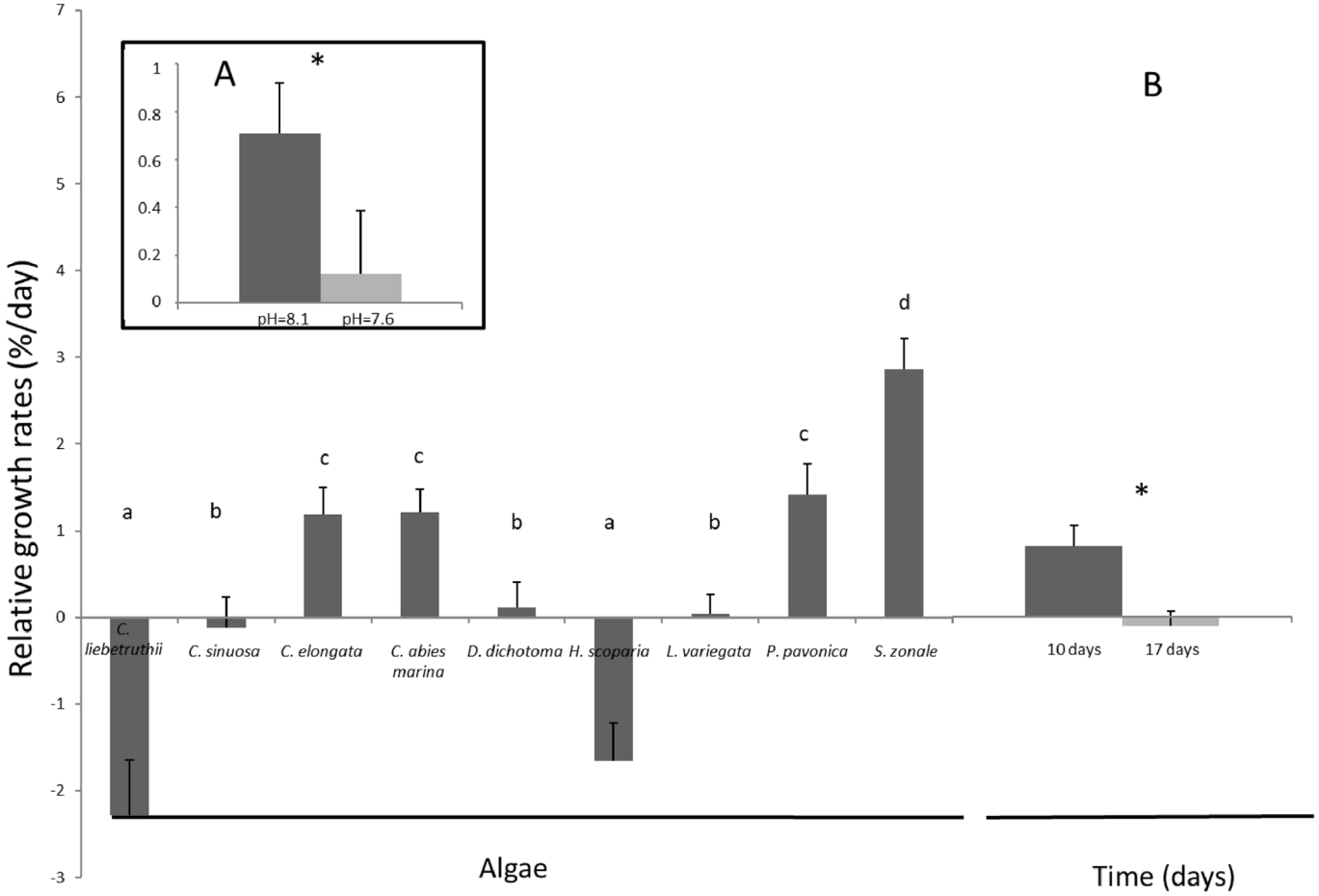


**Fig. 3.** Mean values ( ± SD) of relative growth rate (g/day) of algae in general in (A) both pH treatments (Control pH = 8.1 and Low pH = 7.6) and **(B)** for each species of algae assessed in the laboratory experiment. The summary bar graph (far right) shows the mean growth for the two exposure times (10 days and 17 days respectively). Differences significatives are showed with asterisk (*) between pH treatments and exposure times and with different letters between algae species.

**Table 5**
Results of two-way permutational ANOVA comparing algae decalcification between pH treatments (pH = 8.1 vs. pH = 7.6) and algae species (*C. liebetruthii*; *C. elongata*; *C. abies-marina*; *D. dichotoma*; *H. scoparia*; *L. variegata*; *P. pavonica*; *S. zonale*).

| Source of variation | df | SS | MS | Pseudo-F | p (perm) |
|---|---|---|---|---|---|
| *Treatment pH(Tr)* | 1 | 1043 | 1043 | 24.06 | **0.001** |
| *Algae (Al)* | 7 | 4848.1 | 692.58 | 15.98 | **0.001** |
| *Tr* Al* | 7 | 590.11 | 84.301 | 1.94 | 0.093 |
| *Residual* | 32 | 1387.1 | 43.346 | | |
| *Total* | 47 | 7868.3 | | | |

2016) were inhibited at seawater pH < 8.0. However other studies show an increase in growth rates in algae under low pH conditions, as response of tolerance to the ocean acidification, for instance Roleda and Hurd (2012) recorded a higher growth rate of *L. variegata* in low pH values close to 7.6.

Problems related with decalcification have been observed by other authors in *P. pavonica* (Johnson et al., 2012; Betancor et al., 2013) and *E. elongata* (Martin and Gattuso, 2009) at low pH levels. Despite the general tendency toward decalcification of algae under acidification (Gao et al., 1993; Cao and Caldeira, 2008; Hofmann et al., 2012), laboratory studies with *E. elongata* showed no change in the carbonate content between different pH treatments (Egilsdottir et al., 2012). These authors suggested that this could be due to features inherent to the collection site; at intertidal areas, algae are continuously subject to wide daily pH fluctuations. Therefore, depending on the coastal band, subtidal or intertidal, algae may show different responses to acidification (Egilsdottir et al., 2012), as observed in other organisms (Bray et al., 2014). However, the decalcification observed in this study in non-carbonated and carbonated species has only been related with changes in feeding rates of *P. lividus*, which increased on algae reared at low pH. This might decrease alga cover if the herbivore species are benefit from ocean acidification, as could be happen with *P. lividus* juveniles from Canary Islands, where studies highlights the adaptation of intertidal species, such as *P. lividus*, to ocean acidification conditions (García et al., 2018a, 2018b).

In conclusion, our results demonstrate that the levels of ocean acidification expected in a future scenario of climate change will affect algae growth. The observed changes in feeding rates, especially in juveniles of *P. lividus*, could significantly promote a shift in algal assemblages. Indirect effects, such as increases in feeding rates for *P. lividus* under low pH conditions, have been shown to be important, as other studies have previously revealed in other invertebrates (Burnell et al., 2013, Poore et al., 2013, 2016). Any shift in palatability of algae is likely to have a strong influence on the structure of coastal systems. Additional research is required to better predict and address changes in secondary metabolites and nutritional contents, as change in algae palatability, of these algae species under different pH levels. Energy costs of juvenile sea urchins also need to be evaluated in future climate change scenarios, and an integrative of multiple choice experiments with multiple factors such as eutrophicazion and warmer are necessary to know the indirects effects of climate change on relationship algae-sea urchins.

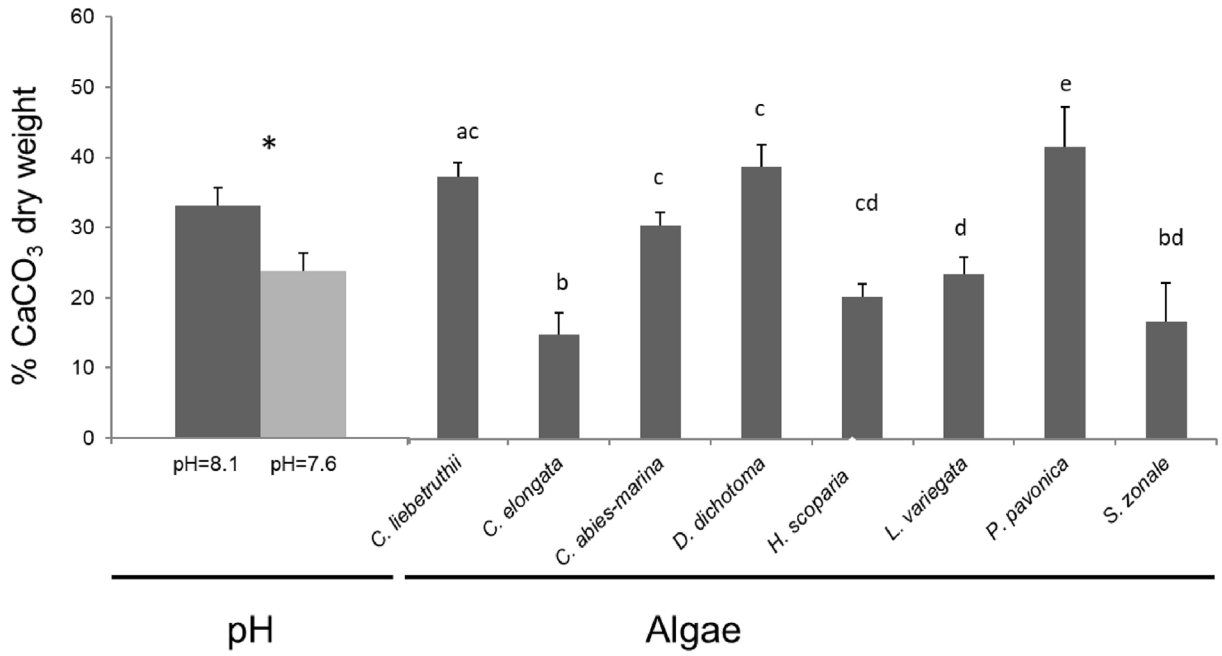


**Fig. 4.** Mean values ( ± SD) of percentage of $CaCO_3$ content in each algae species and at the two pH treatments (Control pH = 8.1 and Low pH = 7.6) in the laboratory experiment. Differences significatives are showed with asterisk (*) between pH treatments and with different letters between algae species.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.marenvres.2018.07.004.

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